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ORIGINAL

Analysis of Paraben Preservatives in Cosmetic Samples: Comparison of Three Different Dynamic Hollow Fiber Liquid-Phase Microextraction Methods

Ali Esrafili · Yadollah Yamini · Mahnaz Ghambarian · Morteza Moradi

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Abstract This study focused on a comparison of three different dynamic hollow fiber-based liquid-phase microextraction (DHF-LPME) methods for extraction and preconcentration of parabens from wastewater, toothpaste, cream, and shampoo samples. The first method is two-phase DHF-LPME, in which *n*-octanol was used as the extraction solvent. The second is three-phase DHF-LPME, in which *n*-octanol and basic aqueous solution were used as the extraction solvent and acceptor phase, respectively. High-performance liquid chromatography with UV detection (HPLC–UV) was applied for determination of the parabens in both methods. The third method is a recently introduced method; three-phase DHF-LPME based on two immiscible organic solvents (*n*-dodecane as organic solvent and acetonitrile as an acceptor phase). The quantitative analyses were performed by the use of gas chromatography-mass spectrometry (GC–MS) after injection port derivatization. The effect of different extraction conditions (i.e., extraction solvent, pH, ionic strength, stirring rate, and dynamic and extraction times) on the extraction efficiency of the parabens was investigated and optimized. All the three procedures provide similar working parameters characterized by high repeatability (3.9–6.3 %) and good linearity (correlation coefficient ranging from 0.989 to 0.998). Results of real sample analyses obtained by these three methods were highly correlated. Although all methods provide compatible alternatives for paraben analysis, the three-phase DHF-LPME based on two immiscible organic solvents may be a more appropriate technique due to its higher extraction efficiency and thus lower limits of detection (LODs). LODs for all the parabens ranged from 0.2 to 5.0 $\mu g\ L^{-1}$ using the two first methods combined with HPLC–UV. An improvement in sensitivity of several orders of magnitude was achieved using three-phase DHF-LPME based on two immiscible organic solvents followed by single-ion monitoring GC–MS analyses (0.01–0.2 $\mu g\ L^{-1}$) due to compatibility of this technique with GC instrument.

Keywords Dynamic hollow fiber-based liquid-phase microextraction · High-performance liquid chromatography · Gas chromatography-mass spectrometry · Parabens

A. Esrafili
Department of Environmental Health Engineering, School of Public Health, Iran University of Medical Sciences, Tehran, Iran

Y. Yamini (✉)
Department of Chemistry, Faculty of Sciences, Tarbiat Modares University, P.O. Box: 14115-175, Tehran, Iran
e-mail: yyamini@modares.ac.ir

M. Ghambarian
Iranian Research and Development Center for Chemical Industries, ACECR, P.O. Box 13145-1494, Tehran, Iran

M. Moradi
Department of Semiconductors, Materials and Energy Research Center, Karaj, Iran

Introduction

The oldest and most basic sample preparation method is extraction, in which the analyst aims to separate the analyte of interest from a sample matrix using a solvent, with an optimum yield and selectivity, so that as few potential interfering species as possible are carried through to the analytical separation stage. In the vast majority of cases, liquid–liquid extraction (LLE) has been the primary sample preparation method to achieve this objective, and is still very popular. However, LLE methods are time consuming

and tedious, and utilize large amounts of high purity organic solvents, which are potentially toxic and expensive. Another popular sample preparation procedure is solid-phase extraction (SPE), introduced commercially in the late 1970s [1]. Consumption of organic solvents is relatively low in SPE, but to obtain a high preconcentration factor, evaporation of the eluent after extraction is required. There have been substantial efforts in the past two decades to adapt the existing sample preparation methods and develop new approaches to save time, labor, and materials. This progress has been very important in the development of novel approaches resulting in new trends in sample preparation, for example, microextraction and miniaturization of sampling and separation steps of the analytical process.

Miniaturized LLE, or liquid-phase microextraction (LPME), was introduced in 1996, in which extraction normally takes place in a small amount of a water immiscible solvent (sometimes referred to as the acceptor phase). The volume of the acceptor phase is in the microliter region. High enrichment factors are achievable because of the high ratio of sample volume to acceptor phase volume. LPME is simple to implement, generally fast, and is characterized by its affordability and reliance on widely available apparatus or materials. In the simplest form of LPME, the organic solvent droplet is held at the tip of a microsyringe needle and is directly immersed in the sample. Since the extraction medium is in the form of a droplet, this implementation of LPME has been separately termed as direct immersion of single drop microextraction (DI-SDME). The major problem of the technique is that the microdrop suspended on the microsyringe needle is easily dislodged during stirring of the aqueous sample, although the selection of a syringe with a beveled needle tip and a very small volume of solvent can obviate this difficulty. Furthermore, the technique is not suitable for dirty samples, because particles in the sample affect the extraction by making the drop unstable, and are potentially detrimental to the analytical instrument [2]. As an attempt to improve the stability and reliability of LPME, Pedersen-Bjergaard and Rasmussen introduced a hollow fiber-based LPME (HF-LPME) in 1999 [3], where the extracting phase was placed inside the lumen of porous hollow fibers made of polypropylene. In this HF-LPME system, target analytes extract from an aqueous sample into the organic solvent immobilized as a thin supported liquid membrane (SLM) located inside the pores of the wall of a porous hollow fiber, and into the acceptor solution placed inside the lumen of the hollow fiber. This method could be performed either in the two- or three-phase mode. In the two-phase mode, the organic solvent presents both in the porous wall and inside the lumen of the hollow fiber [4–11]. In the three-phase mode, the acceptor phase can be aqueous resulting in a conventional three-phase system compatible with high-performance liquid chromatography or capillary electrophoresis [12–16], or the acceptor solution can be organic providing a three-phase extraction system with two immiscible organic solvents compatible with all instruments without any limitation [17–19]. In another similar work, Basheer et al. [20] reported a three-phase mode of HF-LPME that involved using an immiscible ionic liquid and organic solvent as SLM and acceptor phase, respectively.

It appears that the hollow fiber decelerates the process of organic solvent dissolution into the bulk solution. The disposable nature of the hollow fiber totally eliminates the possibility of sample carryover and ensures reproducibility. In addition, the small pore size prevents entering large molecules and particles present in the donor solution into the acceptor phase. At the same time, most existing components in the solution do not enter the hollow fiber because of their very low solubility in the organic phase present in the pores; thus, yielding very clean extracts. Thus, HF-LPME is a more robust and reliable alternative for LPME. In addition, the needed equipments are very simple, inexpensive, and also offer good possibilities for automation in comparison with other LPME methods.

The aim of the present study was to compare efficiency of the three aforementioned HF-LPME methods as a dynamic model for extraction of parabens as model compounds from various real samples such as wastewater, toothpaste, shampoo, and cream. The quantitative analyses for extracts resulted from two-phase and conventional three-phase DHF-LPME (aqueous acceptor phase) methods were performed by HPLC–UV instrument. Furthermore, analysis of the extracts resulted from three-phase DHF-LPME method based on two immiscible organic solvents was carried out using GC–MS instrument.

Experimental

Chemicals and Supplies

The Accurel Q3/2 polypropylene hollow fiber membrane (600 μm i.d., 200-μm wall thickness, and 0.2-μm pore size) was supplied by Membrana (Wuppertal, Germany). 4-Hydroxybenzoic acid, methylparaben, ethylparaben, and propylparaben were purchased from Aldrich (Milwaukee, WI, USA). 1-Octanol, *n*-dodecane, dihexyl ether, 1-undecanol, trioctylphosphine oxide (TOPO), methanol, acetonitrile, ammonium acetate, and sodium chloride with the highest purity were supplied by Merck (Darmstadt, Germany). Stock standard solutions of each analyte with concentration of 1,000 mg L^{-1} were prepared separately in methanol and stored at 4 °C. Mixtures of working standard solutions with different concentrations were prepared daily by dilution of stock solutions with methanol. Mixtures of

standard working solutions for extraction at different concentrations were prepared by dilution with water purified by a Milli-Q water purification system from the Millipore Company (Bedford, MA, USA). Before extraction of the parabens, the cosmetic samples including shampoo, cream, and toothpaste samples were properly diluted with ultrapure water and wastewater samples were filtered through a 0.45 μm pore size cellulose acetate membrane filters.

Instrumentation

The HPLC system consisted of a Varian 9012 HPLC pump (Mulgrave Victoria, Australia), an injector equipped with a 20-μL sample loop, a Varian 9050 UV/Vis detector. Chromatographic data were recorded and analyzed using a home designed computerized software. Separations were carried out on a PerfectSil Target ODS column (250 × 4.6 mm, with 5-μm particle size) from MZ-Analysen Technik GMBH (Wöhlerstraße, Germany). A mixture of 50 mM ammonium acetate (CH_3COONH_4) buffer solution (pH 4.0) and acetonitrile (57:43) at a flow rate of 1 mL min^{-1} was used as a mobile phase in isocratic elution mode. Injection volume was 20 μL for all the samples and detection was performed at the wavelength of 254 nm. A 25-μL (model 702 N) and 50-μL (model 1705 N) Hamilton microsyringes (Bonaduz, Switzerland) were employed for injection and extraction, respectively.

The gas chromatographic system comprised an Agilent (Centerville Road, Wilmington, USA) series 7890A GC coupled to an Agilent MSD 5975C quadrupole mass spectrometer. The GC was fitted with HP-5 MS capillary column (30 × 0.25 mm i.d., 0.25-μm film thickness) from Agilent J&W Scientific (Folsom, CA, USA). Helium (99.999 %) was used as the carrier gas at the flow rate of 1.0 mL min^{-1}. The following temperature program was employed for the separation: 70 °C for 1 min, increased to 200 °C at 10 °C min^{-1}, and held for 1 min; finally increased to 300 °C at 50 °C min^{-1}. The MS quadrupole and the MS source temperatures were set at 150 and 230 °C, respectively. Data acquisition was performed in the full scan mode (m/z in the range of 50–700) to confirm the retention times of analytes and in selected ion monitoring (SIM) mode for quantitative determination of parabens. A dwell time of 100 ms was used for each mass operated in SIM mode with high resolution. The filament delay time was set at 3 min. The monitored ion was 121 m/z for all parabens. The injection volume to GC–MS instrument was 1 μL.

A homemade programmable syringe pump was employed for dynamic extraction process and a magnetic stirrer/hot plate from Heidolph (Kelheim, Germany) was applied for stirring of the solutions.

Extraction Procedure

The experimental setup is illustrated in Fig. 1. Twenty mL of aqueous sample containing 100 μg L^{-1} of parabens was placed in a 21-mL sample vial, which was placed on a magnetic stirrer plate. A 12 × 4-mm magnetic stirring bar was placed in the donor solution to ensure efficient stirring during the extraction. A 25-μL HPLC syringe and a conventional medical syringe needle were inserted through the silicon septum; the former served to introduce the acceptor solution into the hollow fiber prior to extraction and to collect this solution after extraction, while the latter was utilized for supporting the hollow fiber.

Briefly, dynamic HF-LPME consists of the following steps: The hollow fiber was cut into 8-cm segments. Prior to use, the segments were sonicated in HPLC grade acetone for 10 min to remove any contaminants in the fiber. They were subsequently removed from acetone and dried in the air. The fiber was immersed in the extracting organic solvent for several seconds to impregnate the pores of the fiber with extracting solvent. In the two-phase HF-LPME, the pores and lumen of hollow fiber were filled by the same organic solvent (1-octanol), but in the three-phase HF-LPME, after solvent impregnation (*n*-dodecane), the solvent in the lumen of fiber was removed by air blowing from a 5-mL medical syringe. A 25-μL portion of the organic acceptor solvent (acetonitrile) or water acceptor phase was withdrawn into the microsyringe and the needle tip was inserted into one end of the hollow fiber. The other end of the hollow fiber was connected to the needle of the conventional medical syringe to support the fiber. The assembly was immersed in the sample solution and the microsyringe was fixed on the programmable syringe pump. The magnetic stirrer and the programmable syringe pump were then simultaneously switched on. The stirring speed was set at 800 rpm for all extractions.

The plunger was depressed at a speed of 3 μL/s to fill all the acceptor solvent into the hollow fiber. After a preset waiting time, the plunger was withdrawn at the same speed to discharge the fiber from acceptor solvent. The above cycles were then repeated for a prescribed number. The programmable syringe pump and the stirrer were switched off at the end of the extraction time. The acceptor solvent in the fiber was withdrawn back into the microsyringe and then flushed into a microtube (100 μL) with a conical bottom and then analyzed by HPLC and GC–MS instruments.

Injection Port Derivatization of 4-Hydroxybenzoic Acid

Derivatization step is required to improve GC–MS resolution and peak shape of 4-hydroxybenzoic acid for GC–MS analysis. The procedure is initiated by reacting the carboxylic acid group with tetrabutyl ammonium

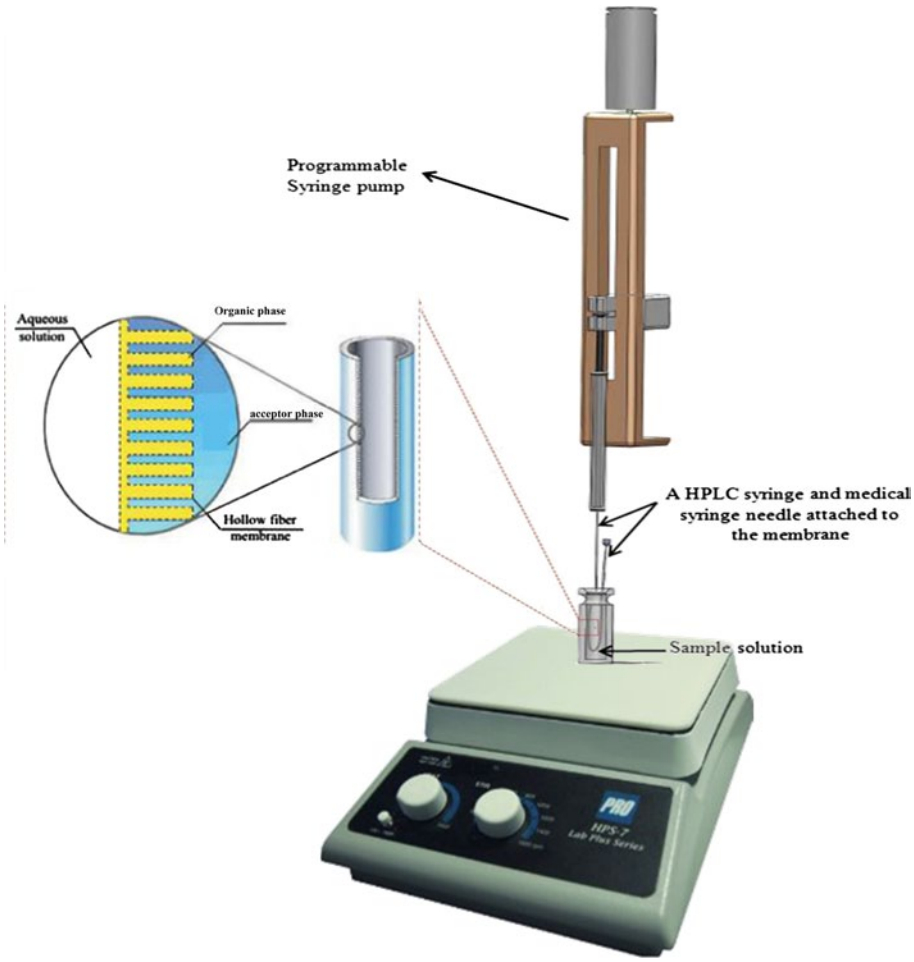

Fig. 1 Schematic diagram of the proposed HF-LPME

bromide $[N(Bu)_4^+ Br^-]$ to form carboxylate ion pairs $[RCOO^-N(Bu)_4^+]$ in solution. Upon introduction to a high temperature GC injection port, the ion pairs are transformed to their corresponding volatile butylesters [RCOOBu]. The derivatization reaction and effect of tetrabutylammonium bromide in derivatization efficiency are shown in Fig. 2. Based on the results obtained, the tetrabutylammonium bromide with a concentration of 500 mg L^{-1} was used for derivatization of 4-hydroxybenzoic acid.

Results and Discussion

To optimize the parameters affecting the three different dynamic HF-LPME methods, the parameters were divided into common and specific parameters. The effects of stirring rate, ionic strength, pH of donor phase, and type of extraction solvent were investigated as common effective parameters on extraction efficiency of all proposed methods. On the other hand, the effect of pH of acceptor phase, and dwelling and extraction times were separately studied as specific parameters for each dynamic HF-LPME method. All of the experiments were repeated at least three times to obtain suitable precision.

Common Parameters

Extraction Solvent

The type of organic solvent used in HF-LPME was an essential consideration for successful experiments. The criteria for selection of an appropriate organic solvent include high partition coefficients of the analytes in the organic solvent, low volatility to prevent solvent loss, immiscibility with water to prevent leakage, the solvent compatibility with hollow fiber, low viscosity to ensure high diffusion coefficients across the SLM, and no or low toxicity. Considering the above issues, some organic solvents, namely *n*-octanol, dihexyl ether, 1-undecanol, *n*-dodecane, and solution of *n*-dodecane + 10 %TOPO were evaluated as the extraction solvents for DHF-LPME. According to the results (Fig. 3), *n*-octanol was selected for two-phase DHF-LPME and conventional three-phase DHF-LPME with aqueous acceptor phase and mixed solvents of

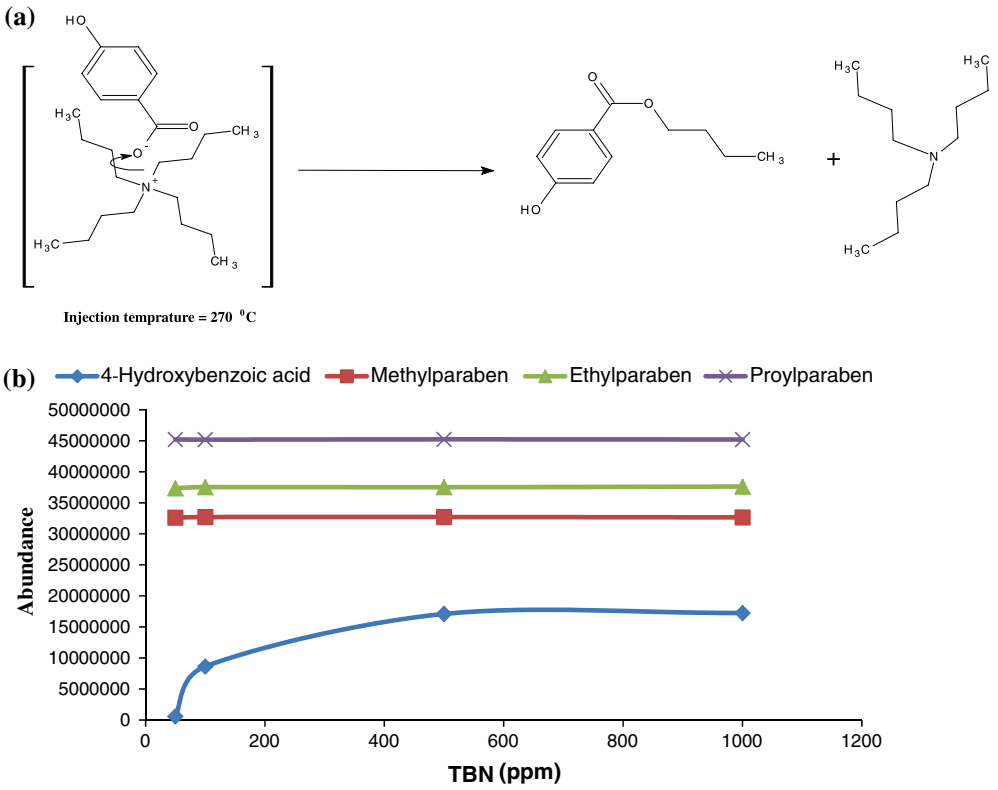

Fig. 2 **a** Derivatization reaction of 4-hydroxybenzoic acid. **b** Effect of tetrabutylammonium bromide concentration on derivatization efficiency of 4-hydroxybenzoic acid

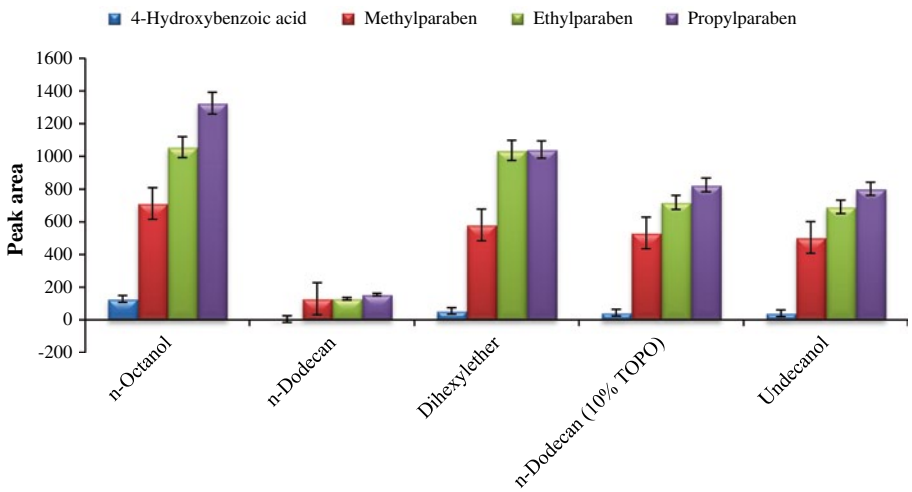

Fig. 3 The effect of type of organic solvent used in HF-LPME on extraction efficiency of the parabens

n-dodecane + 10 % TOPO were chosen for three-phase DHF-LPME based on two immiscible organic phases.

Ionic Strength

Addition of salt to the sample solution may have several effects on the extraction efficiency of paraben compounds. Extraction efficiency could be enhanced by the addition of salt due to decreasing the solubility of analysts in the aqueous sample and increasing their partitioning into the organic phase (salting-out effect). It is assumed that apart from the salting-out effect, the presence of salt could have a second effect and change the physical properties of the Nernst diffusion film; thus, reducing the rate of diffusion of the target analytes into the solvent layer on the hollow fiber. The effect of NaCl concentration (in the range of 0–20 %

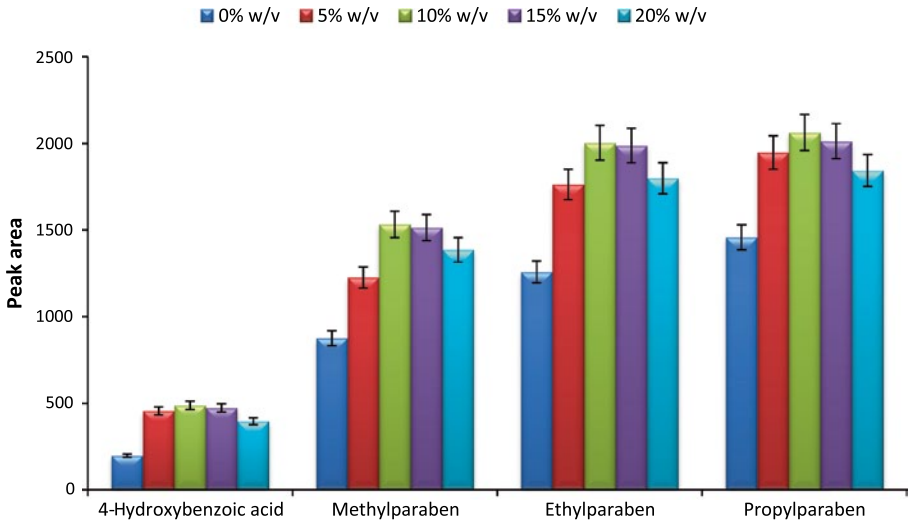

Fig. 4 Effect of NaCl concentration on the relative peak area of parabens

w/v) was investigated and the extraction efficiencies were monitored. As shown in Fig. 4, the extraction efficiencies reached a maximum at 10 % w/v of sodium chloride and subsequently decreased slowly with the salt concentration up to 20 % w/v. Based on these observations, the salt concentration of 10 % w/v was used for further studies.

pH of Donor Phase

According to structures of the parabens, to extract 4-hydroxybenzoic acid, as a weak acid, into the organic phase, the pH of the donor phase was acidified to convert the analytes into undissociated form. The effect of pH in the range of 1.0–9.0 was investigated. Extraction efficiencies of methylparaben, ethylparaben, and phenyl paraben were not significantly affected by pH, while that of 4-hydroxybenzoic acid remained constant when pH was increased from 1.00–2.00 and then dramatically decreased by further increasing of the pH value. The pK_a value of 4-hydroxybenzoic acid is 4.48. Theoretically, a donor phase pH value of 2.00 (equal to $pK_a - 2$) would be sufficiently acidic. Therefore, the pH value of 2.0 was chosen as the optimum pH value for extraction.

Stirring Rate

In HF-LPME, the extraction can be accelerated by stirring or sonicating the aqueous solution, because the agitation permits continuous exposure of the extraction surface to fresh aqueous sample. In this study, the stirring speed was optimized to obtain the highest extraction performance. The experimental results supported this explanation. The extraction performances increased with the increase of stirring speed from 200 to 1,000 rpm. By further increasing of stirring speed than 1,000 rpm, excessive air bubbles were generated that could adhere to the hollow fiber surface. The attached air bubbles appeared to promote solvent evaporation since the observation indicated that solvent loss was faster and greater with respect to the absence of air bubbles. In this condition, precision was poor. Therefore, 1,000 rpm was selected as the optimum stirring rate.

Specific Parameters

The pH of Acceptor Phase

The preconcentration was more sensitive to compositional (pH) variations of the acceptor solution. In basic solution, the acid–base equilibrium for the acidic compounds significantly shifts toward the ionic form, which has greater affinities toward the acceptor phase and the extraction efficiencies are, therefore, increased. The effect of the acceptor basicity on the extraction efficiency was studied by changing the NaOH concentration from 10^{-7} to 1.0 M in each of the three-phase hollow fiber microextraction methods with aqueous (Fig. 5a) and methanol acceptor phases (Fig. 5b). The results indicate that the extraction efficiency increases by increasing NaOH concentration, reaching a maximum at a concentration of 0.01 mol L^{-1} NaOH, and remains almost constant in higher concentration except for 4-hydroxybenzoic acid in which it slightly decreases. Therefore, the concentration of 0.01 mol L^{-1} NaOH was selected for both aqueous and organic acceptor phases in subsequent experiments.

Extraction Time

Like other microextraction techniques, HF-LPME is a type of equilibrium, rather than exhaustive, extraction technique. A period of time is, therefore, required for the equilibrium to be established. The effect of exposure time on extraction efficiency was evaluated for all the three methods.

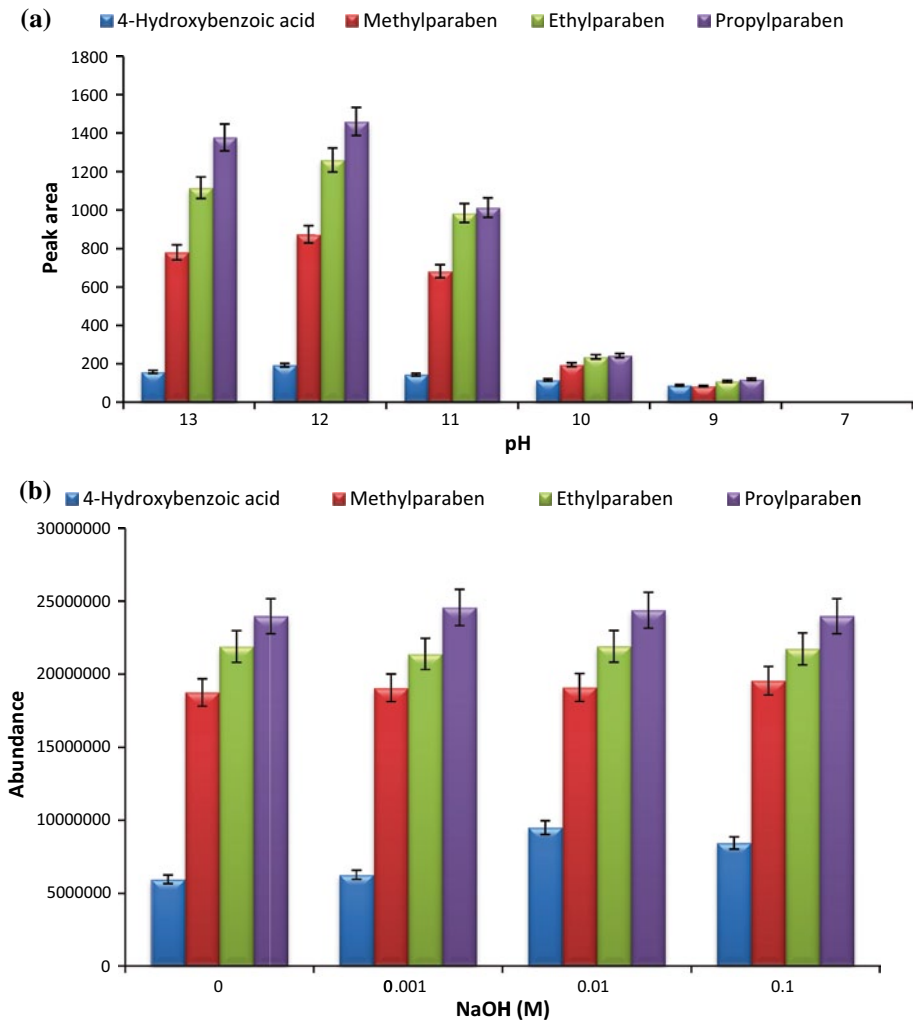

Fig. 5 Effect of NaOH concentration in acceptor phases on the extraction efficiency of three-phase HF-LPME methods **a** with aqueous acceptor phase **b** with organic acceptor phase

Extractions were conducted for 10, 20, 30, 40, and 50 min at a stirring rate of 1,000 rpm. The extraction efficiency for the target compounds was found to increase rapidly by increasing the exposure time from 10 to 30 min. It also increased, but at a slower rate, between the extraction times of 30 and 50 min. Therefore, a sample extraction time of 40 min was chosen for subsequent studies.

Dwelling Time

In the dynamic process, extraction is performed by automatically manipulating the plunger in and out of the microsyringe barrel. Dwell time is described as the time between refilling and infusing of the organic extraction solvent within the lumen of hollow fiber, which is an important factor for the repeated plunger movement. The shorter the dwell time is, the higher the frequency of the plunger movement is. This in turn allows a greater number of extraction cycles. The higher frequency of the plunger movement was beneficial to mass transfer; however, short dwelling time may result in shorter contact time between the acceptor phase in lumen and organic solvent layer in the pores of the hollow fiber. To investigate the effect of dwelling time on extraction efficiency, the plunger speed was kept at 3 $\mu L\ s^{-1}$ and the dwelling time was varied in the range of 0–5 min. When the dwelling time varied from 0 to 2 min, extraction efficiency increased, and longer dwell times yielded a decrease in the extraction efficiency. For practical reasons, 2 min was chosen as the dwelling time for the rest of the study.

Method Evaluation

For method comparison, LOD, preconcentration factor, and repeatability of the individual methods were measured and tabulated in Table 1. For all the methods, calibration curves have been constructed using a least square linear regression analysis of standard mixtures of the analytes in the range of 1–100 $\mu g\ L^{-1}$. The best coefficients of determination to straight lines were obtained for the

Table 1 Figures of merit of three different HF-LPME methods for extraction of parabens from water samples

Method	Analyte	LDR (μg L^{-1})	LOD (μg L^{-1})	R^2	PF	RSD %	ER %
Two-phase DHF-LPME[a]	4-Hydroxybenzoic acid	10–100	5	0.994	30.0	6.3	3.0
	Methylparaben	5–100	2	0.989	117	4.1	11.7
	Ethylparaben	5–100	2	0.993	143	4.6	14.3
	Propylparaben	5–100	2	0.988	162	4.7	16.2
Three-phase DHF-LPME with aqueous acceptor[a]	4-Hydroxybenzoic acid	5–100	2	0.992	58	5.8	5.8
	Methylparaben	1–100	0.5	0.998	215	4.3	21.5
	Ethylparaben	1–100	0.5	0.993	295	4.7	29.5
	Propylparaben	1–100	0.5	0.993	285	5.3	28.5
Three-phase DHF-LPME with organic acceptor[b]	4-Hydroxybenzoic acid	0.5–100	0.2	0.995	84	6.0	8.4
	Methylparaben	0.1–100	0.05	0.999	302	3.9	30.2
	Ethylparaben	0.1–100	0.05	0.998	317	5.1	31.7
	Propylparaben	0.07–100	0.01	0.996	313	4.7	31.3

[a] Analyzed by HPLC–UV

[b] Analyzed by GC–MS

three-phase DHF-LPME based on organic acceptor phase (>0.995). These coefficients for three-phase DHF-LPME with aqueous acceptor phase were >0.992, and for two-phase DHF-LPME for all compounds were >0.989. The results obtained show that all the methods are characterized by high linearity in the examined concentration ranges. The LODs (based on $S/N = 3$) for paraben compounds were obtained in the range of 2–5 μg L^{-1} for two-phase DHF-LPME, 0.5–2 μg L^{-1} for conventional three-phase HF-LPME, and 0.01–0.2 μg L^{-1} for three-phase DHF-LPME based on using two immiscible organic solvents. An improvement in detection limits was achieved for the latter method using GC–MS analyses, owing to the compatibility of this technique with gas chromatography instrument.

Repeatability of the methods was examined by five times replicate extraction and determination of the analytes using different extraction methods. The results demonstrated that the three methods are not significantly different. The procedures had good repeatability for paraben compounds. Relative standard deviations (RSD) ranged from 4.1 to 6.3 % for two-phase DHF-LPME, from 4.3 to 5.8 % for conventional three-phase DHF-LPME, and from 3.9 to 6.0 % for three-phase HF-LPME based on using two immiscible organic solvents. Determination of 4-hydroxybenzoic acid indicated a slightly inferior repeatability, because another derivatization step was required before extraction. For 20 mL sample solution, the preconcentration factors (PFs) obtained from two and three-phase DHF-LPME with aqueous and organic acceptors were in the range of 33–162, 58–295, and 84–317, respectively, which related to an extraction recoveries of 3.0–31.3 %.

Figure 6 shows a typical chromatogram of the parabens extracted from water sample using the three proposed DHF-LPME methods. In conclusion, the results obtained revealed that the three techniques have high sensitivity and they are suitable for the quantitative analysis of parabens in cosmetic samples. In general, the three-phase DHF-LPME method based on using two immiscible organic solvent provides better figures of merits compared with the two other methods.

Application of the Proposed Methods to Real Samples

The three extraction methods were applied to the extraction and determination of parabens in wastewater, shampoo, cream, and toothpaste samples. Each treatment was in triplicate, the results obtained are provided in Table 2. Accurately weighed amounts of shampoo, cream, and toothpaste samples (1.0 g) were properly diluted in ultrapure water (between 1:20 and 1:100, v/v); then, the sample solutions were treated with ultrasound for 10 min and then centrifuged for 5 min. The pH of the supernatant was adjusted to pH 2 and then the supernatant liquid was subjected to the proposed procedures.

To assess matrix effects, the samples were spiked with 1 and 10 mg kg^{-1} of each analyte. Experiments were conducted under the optimum extraction conditions. The relative recoveries obtained from two- and three-phase DHF-LPME were in the range of 85.6–103.0 %. Figure 7 shows the typical HPLC chromatograms of the extracted parabens using three-phase DHF-LPME based on organic acceptor phase from cream before and after spiking with 10.0, 20.0, 40.0, and 50.0 μg L^{-1} of parabens. Also, Fig. 8 illustrates the GC–MS chromatogram for a wastewater sample, showing the presence of methylparaben, ethylparaben, and 4-hydroxybenzoic acid (derivatized as butyl paraben) in the extracted ion chromatograms.

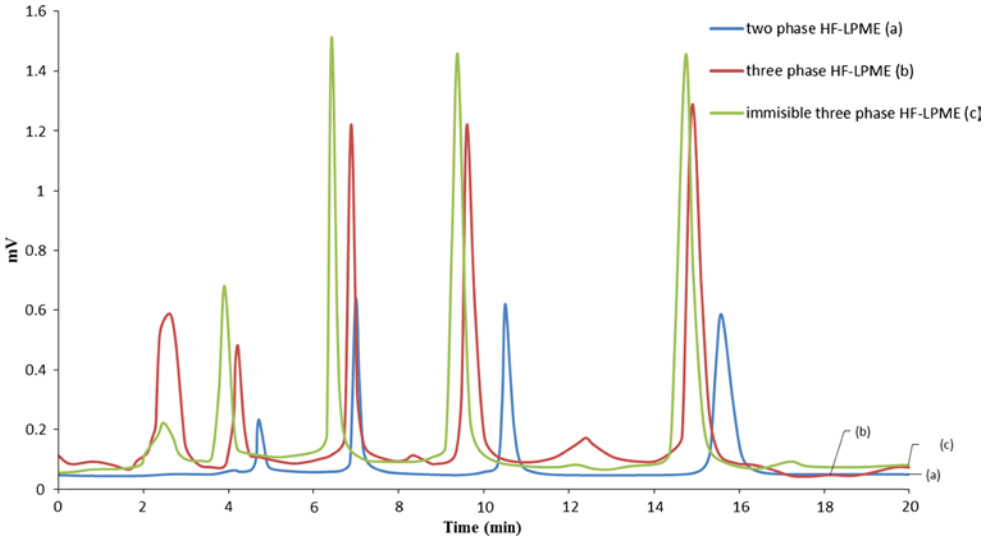

Fig. 6 Comparative chromatograms of the proposed methods for extraction of parabens from water samples

Table 2 Results obtained from analysis of real samples

Real sample	Three-phase DHF-LPME with aqueous acceptor phase				Two-phase DHF-LPME				Three-phase DHF-LPME with organic acceptor phase			
	HBA	MP	EP	PP	HBA	MP	EP	PP	HBA	MP	EP	PP
Shampoo												
Concentration of analytes (mg kg^{-1})	172.6	–	–	7.53	172.6	–	–	7.4	172.6	–	–	8.1
RSD % ($n = 3$)	5.4	–	–	5.5	6.0	–	–	5.9	4.9	–	–	4.8
Cream												
Concentration of analytes (mg kg^{-1})	307.1	7.31	–	4.65	307.1	6.8	–	4.9	307.1	6.7	–	4.2
RSD % ($n = 3$)	6.1	4.5	–	5.5	6.2	5.3	–	5.0	5.8	5.2	–	4.8
Toothpaste												
Concentration of analytes (mg kg^{-1})	514.5	2.6	–	12.8	514.5	2.5	–	13.6	514.5	2.3	–	12.3
RSD % ($n = 3$)	7.0	4.7	–	6.2	8.4	4.5	–	5.5	7.6	4.4	–	6.1
Wastewater 1												
Concentration of analytes (μg L^{-1})	–	–	–	–	–	–	–	–	–	–	–	–
RSD % ($n = 3$)	–	–	–	–	–	–	–	–	–	–	–	–
Wastewater 2												
Concentration of analytes (μg L^{-1})	26.5	–	4.3	–	25.7	–	ND	–	22.8	–	4.7	–
RSD % ($n = 3$)	4.2	–	6.8	–	6.4	–	–	–	5.5	–	4.7	–
Wastewater 3												
Concentration of analytes (μg L^{-1})	47	7.2	ND	–	49.5	6.6	ND	–	45.9	7.0	0.31	–
RSD % ($n = 3$)	5.9	4.6	–	–	6.3	5.0	–	–	6.0	4.7	4.5	–

HBA 4-hydroxybenzoic acid, *MP* methylparaben, *EP* ethylparaben, *PP* propylparaben

Solid-Phase Extraction

Solid-phase extraction (SPE) was applied as an acceptable reference method to confirm the accuracy of the present methods for determination of parabens in the real samples. The parabens were analyzed in 50 mL wastewater using SPE-HPLC procedure. After the C18 SPE column was conditioned according to the manufacturer's recommendations, 50 mL of the sample was passed through the column to extract the parabens. The column was then washed with 2 mL of HCl solution (2 %, v/v) to elute probable interferences. Methanol (4 mL) was used to wash the parabens from the column. The volume of eluate was reduced to 100 μL by nitrogen bubbling. Finally, 20 μL of the residue was injected into the HPLC–UV for analysis. The results are provided in Table 3. The results obtained by the proposed methods were in accordance with those of the reference method and indicated that the quantitative data can be obtained for determination of

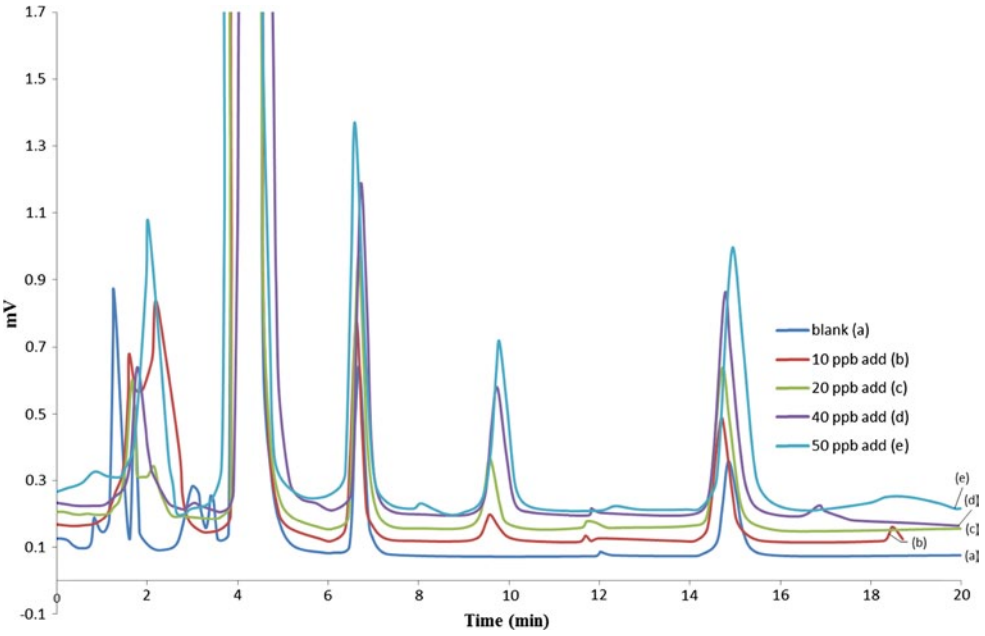

Fig. 7 Chromatograms of the parabens after extraction from the cream sample using three-phase HF-LPME based on using two immiscible organic solvents before and after spiking with 10, 20, 40, and 50 μg L^{-1} of parabens

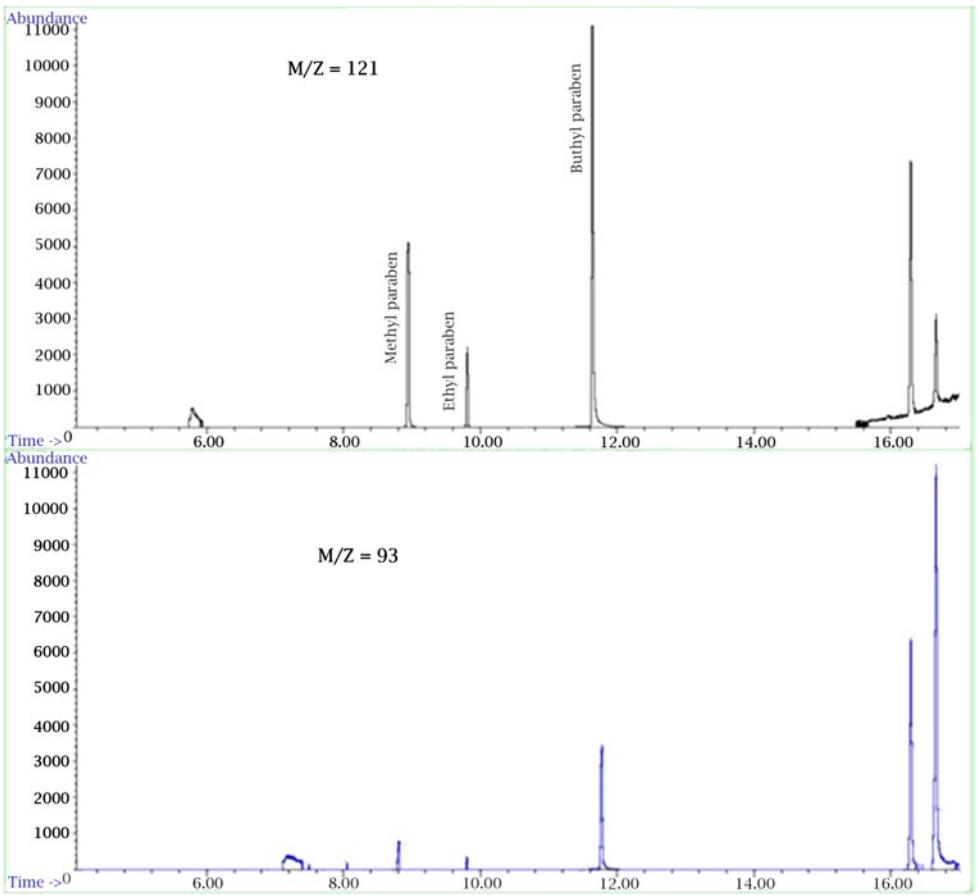

Fig. 8 Extracted ion chromatograms of 93 and 121 m/z from GC–MS chromatogram of parabens after extraction of the wastewater sample using three-phase HF-LPME based on using two immiscible organic solvents

parabens in wastewater samples using HF-LPME procedure. This method has several advantages in comparison with SPE extraction due to reduced solvent volume. Furthermore, it is not necessary in the proposed method to evaporate a large volume of toxic organic solvent which is a time-consuming and inappropriate environmental behavior.

Table 3 Comparison of the proposed methods with the reference method for extraction and determination of the parabens in wastewater 2

Method	HBA	MP	EP	PP
Two-phase DHF-LPME				
Initial concentration ($\mu g\ L^{-1}$)	25.7	–	ND	–
RSD (%) ($n = 3$)	6.4	–	–	–
Three-phase DHF-LPME with aqueous acceptor				
Initial concentration ($\mu g\ L^{-1}$)	26.5	–	4.3	–
RSD (%) ($n = 3$)	4.2	–	6.8	–
Three-phase DHF-LPME with organic acceptor				
Initial concentration ($\mu g\ L^{-1}$)	22.8	–	4.7	–
RSD (%) ($n = 3$)	5.5	–	4.7	–
SPE				
Initial concentration ($\mu g\ L^{-1}$)	25.3	–	4.5	–
RSD (%) ($n = 3$)	6.6	–	5.2	–

Conclusions

The methods of extraction of parabens from cosmetics using three modes of DHF-LPME were established, and the extraction efficiency rates using the three methods were systemically compared. All the methods studied are highly sensitive with low limits of detections and can be successfully applied to separation, preconcentration, and determination of not only parabens, but also other noxious materials in different real samples. The data herein represent the higher efficiency of three-phase DHF-LPME based on two immiscible organic solvents compared to using an aqueous acceptor phase and two-phase DHF-LPME. Features of the method include its simplicity, desirable sensitivity, selectivity and analytical precision, low consumption of organic solvent, low cost, and short sample preparation time. The other compelling analytical feature of the method is its compatibility with GC instruments due to using organic acceptor solvent. Finally, the advantages of hollow fiber-protected LPME allow its potential application as a sample preparation and cleanup technique for drug analysis in biological samples.

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